Plant Physiol. (1978) *62,* 101-104

Physiological Effects of Surface Waxes

I. LIGHT REFLECTANCE FOR GLAUCOUS AND NONGLAUCOUS *PICEA PUNGENS*[1]

Received for publication May 2, 1977 and in revised form March 15, 1978

DAVID A. REICOSKY[2] AND JAMES W. HANOVER
Department of Forestry, Michigan State University, East Lansing, Michigan 48824

ABSTRACT

Foliage reflectance was studied on glaucous and nonglaucous foliage of blue spruce (*Picea pungens* Engel.). Current-year and 1-year-old glaucous and nonglaucous foliage from mature trees and seedling glaucous and nonglaucous foliage had similar reflectance patterns in the 350 to 800 nanometer region. The highest reflectance was in the 750 to 800 nanometer region and the lowest reflectance was in the 670 nanometer region. Glaucous foliage had a higher percentage of light reflectance at all of the wavelengths of light. The largest difference of reflectance between glaucous and nonglaucous foliage was in the 350 nanometer region with a general decline in the difference to the smallest difference at the 800 nanometer region.

The glaucous characteristic of plant leaves, which is due to the light-scattering properties of surface waxes, has been described for many species. Many functions have been proposed for these waxes including regulation of moisture and gas exchange with the atmosphere, protection from mechanical and pest damage, and protection from UV radiation and air pollutants. Despite the potential significance of these and other postulated functions for surface waxes, experimental evidence for specific functions is scarce.

The structure, arrangement, and density of surface waxes largely determine the degree of glaucousness (6, 7). Hanover and Reicosky (7) found variation between species, plants, and individual needles in the quantity and distribution patterns of structural and amorphous wax of six conifer species. They further confirmed Rhine's (10) report that wax rather than soot occurs in the stomatal pits of Austrian pine (*Pinus nigra*) and found considerable variation in the degree of stomatal occlusion by surface waxes.

Structural waxes develop first in the epistomatal chambers of blue spruce needles in expanding buds (9). As needles emerge from the bud structural waxes develop over the entire needle surface on glaucous foliage but are restricted to stomatal areas on nonglaucous foliage. Structural waxes become weathered to an amorphous layer over the entire needle surface with time, but the epistomatal chambers remain occluded with surface waxes. Surface waxes of blue spruce were determined to have an insignificant effect on the boundary layer of the needle (9).

The spectral properties of plants were investigated by Gates *et al.* (5) who found that desert species had higher light reflection values than mesic species and that this represented an adaptation to their respective habitats. However, Sinclair and Thomas (11) reported that although surface reflection of light was higher for desert species, so was light absorption. They concluded that changes in optical properties of leaves were not a significant adaptation to arid conditions. Ehleringer *et al.* (3) have shown that leaf pubescence reduced the absorption of photosynthetically active radiation by 56% and also reduced the net photosynthetic rate of *Encelia* species. A reduction in reflectance of glaucous juvenile leaves of *Eucalyptus bicostata* caused by wiping the leaves with cotton wool increased the rate of photosynthesis at low light intensities but decreased the rate of photosynthesis at high light intensities (1). This indicates that alteration of foliage reflectance by surface waxes may represent an adaptation to certain climatic conditions.

Using relative reflectance values Clark and Lister (2) showed that the low relative efficiency of blue light in photosynthesis of blue spruce foliage is due to reflectance of blue light by epicuticular wax deposits. They also showed differences between treated and untreated blue spruce foliage in relative reflectance in the 200 to 300 nm region of the light spectrum. However, reflection of light of wavelengths shorter than 300 nm has little biological significance since all of the light energy in this region is absorbed by ozone and does not reach the earth's surface (4).

The purpose of this study was to determine the effects of foliage glaucousness on light reflection of blue spruce needles.

MATERIALS AND METHODS

Absolute Light Reflectance. Fully expanded needles were sampled in mid-July from 12 blue spruce (*Picea pungens* Engel.) trees classified as glaucous, semiglaucous, and nonglaucous. Current-year needles of seedlings were also examined. Reflective characteristics of current-year needles, 1-year-old needles, and current-year and 1-year-old needles washed in chloroform were examined in the older trees which ranged in age from 15 to 20 years.

A Zeiss PMQ II spectrophotometer fitted with an integrating sphere was used in the reflectance measurements. Monochromatic light with an incident angle of 90° was shown on the foliage and the diffused reflected light monitored by a photomultiplier tube inside the integrating sphere. The results obtained in this study represent minimum reflectance values since the degree of reflectance increases as the angle of incidence varies from 90° (8).

Needles were mounted as close together as possible on a cardboard square to minimize the effect of light reflected from the cardboard surface. One cm^2 of mounted foliage was placed at one port of the integrating sphere for absolute light reflectance measurements. Per cent reflectance readings were taken at three different orientations for each sample with the mean of the three measurements used for data analysis.

To establish the general pattern of light reflection of blue spruce needles, reflectance measurements of current-year needles of two glaucous and two nonglaucous trees were obtained every 10 nm from 350 to 800 nm. Additional replication and analysis of variance were carried out at 12 wavelengths selected along the established curve.

Epicuticular waxes were removed from current-year and 1-year-

[1] Michigan Agricultural Experiment Station Article No. 8046.
[2] Present address: Agronomy Department, University of Kentucky, Lexington, Kentucky 40506.

old needles of a glaucous and two nonglaucous trees by washing the needles in chloroform for 1 min. Since no differences in reflectance were found between current-year and 1-year-old dewaxed needles from the same tree these reflectance measurements were combined for subsequent analysis.

Electron Micrographs. Fresh needles were affixed to aluminum stubs with Tube Koat cement (Carbon, G.C. Electronics Rockford, Ill.), coated under vacuum with a thin layer of carbon, and then coated with a gold-palladium alloy (60:40) about 200 A thick. Photomicrographs of the samples were obtained with an AMR (Advanced Metals Research) 900 scanning electron microscope at 21 kv.

RESULTS AND DISCUSSION

Distribution of Epicuticular Wax Deposits. Glaucous foliage epicuticular wax deposits are dense and uniformly cover the entire needle surface (Fig. 1). Figure 2 illustrates the structured nature of the epicuticular wax deposits. These waxes are also found on nonglaucous foliage, but they are restricted to the stomatal areas (Fig. 3). The structural waxes of glaucous and nonglaucous needles are completely removed by rinsing in chloroform (Fig. 4). A detailed description of structural waxes of blue spruce is found in references 7 and 9.

Comparison of Absolute Light Reflectance. Glaucous and nonglaucous foliage had the greatest reflectance in the 750 to 800 nm region with values of 70.2 and 65.5%, respectively (Fig. 5). The lowest reflectance values (15.9 and 7.9%, respectively) were found in the 670 nm region. Both glaucous and nonglaucous foliage had an increase of foliar reflectance in the 520 to 570 nm region where little absorption of light by leaf pigments occurs. This general pattern of reflectance is similar for current-year, 1-year-old, and seedling foliage (Table I).

Statistically significant differences were found when the reflectance of current-year needles of glaucous, and nonglaucous trees of blue spruce were compared. Semiglaucous foliage was intermediate in reflectance between the glaucous and nonglaucous foliage but more closely resembled the glaucous type. Glaucous foliage had a higher percentage of light reflectance at all wavelengths of light from 350 to 800 nm. The largest difference of reflectance between glaucous and nonglaucous foliage was in the 350 nm region with a general decline in the difference to the 800 nm region (Fig. 5). The greatest difference of reflectance of glaucous and nonglaucous foliage was in the UV and blue region of the light spectrum where energy per photon is maximum for the wavelengths of light measured. The ability of glaucous foliage to reflect more energy than nonglaucous foliage may be a selective advantage in environments where solar radiation is intense or of long duration. An increase in reflectance should reduce the amount of energy that enters the leaves, and, consequently, the amount of energy that must be dissipated by the leaves.

Removal of the epicuticular waxes, and hence the glaucous characteristic of foliage, by a chloroform rinse (Figs. 1 and 4) greatly reduced the reflectance of the leaf surface and eliminated significant differences in reflectance between glaucous and nonglaucous foliage (Table I). This indicates that a large portion of the observed foliage reflectance was due to the structural waxes. There was a change of foliage reflectance of chloroform-washed foliage with light quality indicating other components of foliage reflectance (Table I). The change of reflectance with light quality is not associated with degrees of glaucousness (Table I) and may be due to the differential absorption of various wavelengths of light by leaf pigments (5).

One-year-old foliage had significantly lower reflectance than current-year foliage but the general pattern of foliage reflectance was similar (Tables I and II). The glaucous appearance of 1-year-

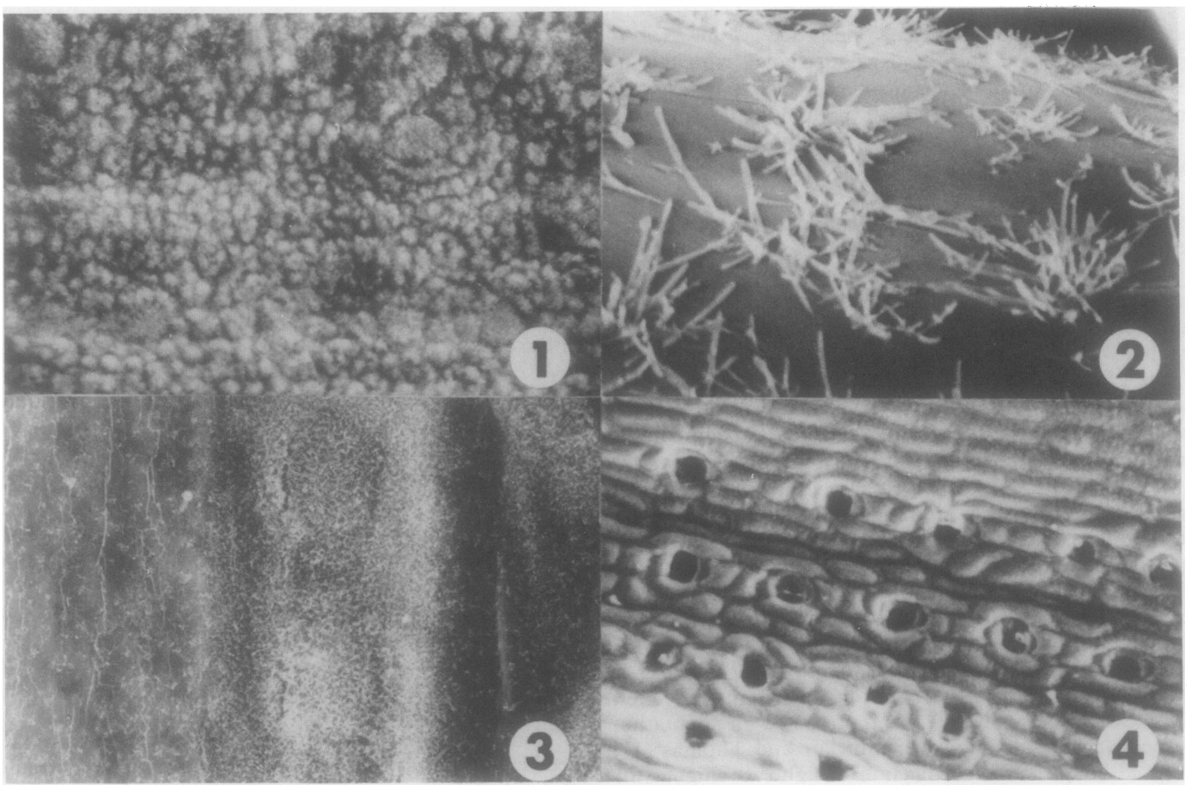

FIG. 1 to 4. Electronmicrographs of blue spruce (*Picea pungens*) needles.
FIG. 1. Glaucous needle with dense epicuticular wax covering (× 200; 1 cm = 68.7 μm).
FIG. 2. Semiglaucous needle with scattered epicuticular wax deposits. Note the structured nature of the wax deposits (× 5,000; 1 cm = 2.75 μm).
FIG. 3. Nonglaucous needle with epicuticular wax deposits only in the stomatal areas (× 500; 1 cm = 27.5 μm).
FIG. 4. Glaucous needle similar to Figure 1 that has been washed in chloroform to remove the epicuticular waxes (× 200; 1 cm = 68.7 μm).

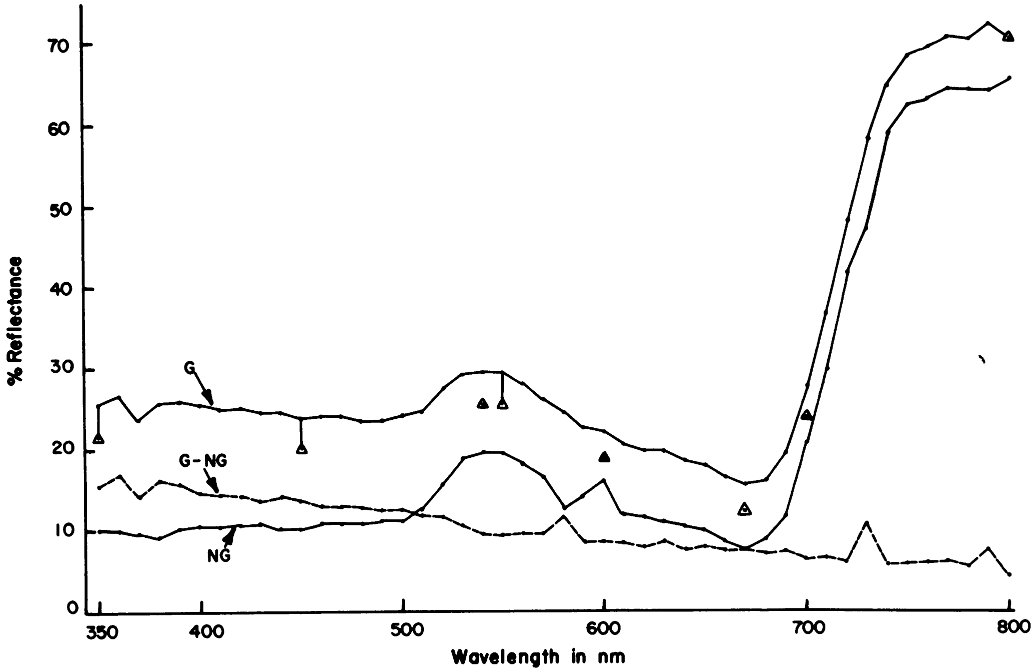

FIG. 5. Reflectance curve of glaucous (G) and nonglaucous (NG) and the difference between these types (G-NG) for current-year needles of blue spruce. Values for semiglaucous determinations are also plotted (Δ). Glaucous and nonglaucous foliage reflectance is significantly different (1% level) at all wavelengths of light. Points not joined by a vertical line indicate significant differences (5% level) of reflectance between the semiglaucous foliage and the other degrees of foliage glaucousness.

Table I. Percent reflectance for glaucous (G) and non-glaucous (NG) needles for current-year, one-year-old, seedling, and chloroform-washed foliage at different wavelengths of light.

Wavelength in nm	Current-year foliage[1/]			One-year-old foliage[1/]			Seedling foliage[1/]			Foliage washed in chloroform	
	Percent reflectance										
	G		NG	G		NG	G		NG	G	NG
350	25.6	**	10.1	15.4	**	6.3	14.4	*	8.5	2.5	2.8
400	-[2/]		-	-		-	-		-	2.9	3.2
450	24.0	**	10.3	15.6	**	7.4	14.5	*	10.2	6.0	5.6
500	-		-	-		-	-		-	8.4	7.9
540	29.6	**	19.8	21.7	**	14.8	21.6		21.3	-	-
550	29.1	**	19.7	21.7	**	14.9	21.4		22.0	14.0	14.0
600	22.4	**	13.5	17.1	**	10.8	15.3		15.2	13.9	12.0
650	-		-	-		-	-		-	9.4	8.4
670	15.9	**	7.9	11.9	**	6.5	9.7	*	7.7	-	-
680	-		-	-		-	-		-	8.3	7.1
700	27.8	**	21.0	23.2	**	17.5	21.9		24.0	-	-
800	70.2	**	65.5	59.3	**	56.4	66.1		65.7	-	-

1/ Significant differences in absolute reflectance values of glaucous and non-glaucous foliage determined by Tukey's test: * = significant at 5% level, ** = significant at 1% level.
2/ Indicates no measurement made.

Table II. Mean percent reflectance for blue spruce needles by wavelength and foliage type. Means followed by the same letter are not significantly different.

Wavelength in nm	Percent reflectance: Current-year foliage		One-year-old foliage		Seedling foliage		Foliage rinsed in chloroform	
350	18.4 a[2/]	A[1/]	10.8 b	A	11.4 b	A	2.7 -[3/]	A
400	-	-	-	-	-	-	3.0 -	A
450	17.6 a	A	11.5 b	A	12.4 b	A	5.8 -	B
500	-	-	-	-	-	-	8.1 -	B,C
540	24.8 a	B	18.2 b	B	21.4 a,b	B	-	
550	24.6 a	B	18.3 b	B	21.7 a,b	B	14.0 -	D
600	18.1 a	A	14.0 b	C	15.3 a,b	C	13.0 -	D
650	-	-	-	-	-	-	8.9 -	C
670	12.0 a	C	9.2 a,b	E	8.7 b	E	-	
680	-	-	-	-	-	-	7.7 -	B,C
700	24.4 a	B	20.4 b	B	23.0 a,b	B	-	
800	68.3 a	D	57.8 b	D	65.4 c	D	-	

1/ Wavelength differences at the 5% level.
2/ Foliage type differences at the 5% level.
3/ Indicates no measurement made.

old blue spruce foliage normally is reduced suggesting that an alteration of light-reflecting properties of leaves may have occurred. Reicosky and Hanover (9) found that changes in structural waxes due to weathering and degradation from a highly structured type in current-year foliage to an amorphous layer in 1-year-old foliage were strongly correlated with the reduction of leaf glaucousness and as shown here light reflectance. Current-year and 1-year-old foliage rinsed in chloroform were not significantly different in foliage reflectance indicating that leaf pigmentation had no effect on foliage reflectance in material of different age. Cameron (1) attributed the reduction of light reflectance of adult leaves of *Eucalyptus bicostata* to a change in density and clustering of the wax rods on the leaf surface. Our results indicate that the change in light reflectance from current-year and 1-year-old foliage is due to the reduction of the highly structured surface waxes to an amorphous type.

Observation of nursery and field plantings indicated an intensification of foliage glaucousness with seedling age. This observation was substantiated by the lower reflectance of current-year needles of seedlings when compared to current-year needles of older trees (Table II). Cameron (1) also found differences in foliage reflectance between juvenile, intermediate, and mature leaves in eucalyptus species. Differences in reflectance of glaucous and nonglaucous foliage of blue spruce seedlings are significant only at 350 nm, 450 nm, and 670 nm (Table II). Lack of significant differences in reflectance at other wavelengths indicates that glaucousness is probably not developed enough at the seedling state to allow complete detection of differences between glaucous and nonglaucous foliage. These results suggest an apparent developmental change which acts to intensify the expression of the glaucous character as the seedling matures. Furthermore, the glaucous character can be expressed only if the genes for wax development are present in the genome.

The glaucous characteristic of blue spruce needles increases light reflectance from the needle surface. Leaf pubescence has been shown to reduce the absorption of photosynthetically active radiation and the net photosynthetic rate of *Encelia* species (4). Clark and Lister (2) have shown that the waxy bloom of blue spruce needles selectively reflects blue light and therefore the needles are less efficient in photosynthesis. Alteration of the surface waxes of *E. biocostata* increased the rate of photosynthesis at low light intensities but decreased the rate of photosynthesis at high light intensities. Energy budget calculations indicate that the glaucous character of leaves may be either a selective advantage or a disadvantage depending on environmental conditions. The reflective properties of epicuticular waxes of glaucous leaves may reduce the energy that is absorbed by the leaf and result in a reduction of leaf temperature. Under conditions of low temperatures (*i.e.* winter conditions) a lower leaf temperature may be disadvantageous due to a reduction of net photosynthetic rate of the leaf. However, a lower leaf temperature could be advantageous under these conditions if conservation of water is important because the glaucous leaf could have smaller transpirational losses. In summer when temperatures are high, a lower leaf temperature could be advantageous due to a reduction in the rate of respiration and a more active photosynthetic process resulting in an increase in net photosynthesis. Transpirational losses may also be reduced by a lower leaf temperature of the glaucous needle especially in hot sunny environments. Although the expected differences in the energy budget of leaves between glaucous and nonglaucous leaves would be small, with time (*i.e.* a growing season or a generation) these factors could have a large and significant additive effect on survival of the individual. Experimental evidence is needed, however, to test the above hypotheses.

LITERATURE CITED

1. Cameron RJ 1970 Light intensity and the growth of *Eucalyptus* seedlings. II. The effect of cuticular waxes on light absorption in leaves of *Eucalyptus* species. Aust J Bot 18: 275-284
2. Clark JB, GR Lister 1975 Photosynthetic action spectra of trees. I. The relationship of cuticle structure to the visible and ultraviolet spectral properties of needles from four coniferous species. Plant Physiol 55: 407-413
3. Ehleringer J, O Björkman, HA Mooney 1976 Leaf pubescence: effects on absorptance and photosynthesis in a desert shrub. Science 192: 376-377
4. Gast PR 1960 Solar radiation. *In* Handbook of Geophysics, Revised Ed, Chap 16 (USAF). Macmillan, New York
5. Gates DM, HJ Keegan, JC Schleter, VR Weidner 1965 Spectral properties of plants. Appl Optics 4: 11-20
6. Hall DM, AI Matus, JA Lamberton, HM Barber 1965 Infra specific variation in wax on leaf surfaces. Aust J Biol Sci 18: 323-332
7. Hanover JW, DA Reicosky 1971 Surface wax deposits on foliage of *Picea pungens* and other conifers. Am J Bot 58: 681-687
8. Howard JA 1967 Spectral energy relations of isobilateral leaves. Aust J Biol Sci. 19: 757-766
9. Reicosky DA, JW Hanover 1976 Seasonal changes in leaf surface waxes of *Picea pungens*. Am J Bot 63: 449-456
10. Rhine JB 1924 Clogging of stomata of conifers in relation to smoke injury and distribution. Bot Gaz 78: 226-232
11. Sinclair R, DA Thomas 1970 Optical properties of leaves of some species in arid South Australia. Aust J Bot 18: 261-273